New cell line establishment of a cell line from embryos of *Dendrolimus superans* [1)]

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Abstract A new cell line was established from 5-day-old embryonated eggs of *Dendrolimus superans* and has been designated NEAU-Ds-950821.The cell line consists of mixture of cell types, including majority of spherical shaped cells and a few of spindle shaped cells. The cell line has a population of doubling time of 52.6 h. Chromosome analysis revealed typical lepidopteran chromosomes. Isozyme characterization of Esterase showed the patterns were different from other three cell lines (Ms-927311, Xc-920730, and SF-21AE). Virus infectivity tests revealed the cell line can support *D. superans* cytoplasmic polyhedrosis virus.
Key words: *Dendrolimus superans*, Embryonated eggs, Characterization, Cell lines.

Introduction

Cytoplasmic polyhedrosis viruses have been isolated from more than 150 species of insects. Some of them have been used as potential insecticides to control forestry insect pests (Shen Hongjian *et. al.* 1994). Investigators have been interested in viruses as an alternative for chemical insecticides to control forestry insect because of their safety and specificity (Pu 1992). But only a few of these viruses have been commercially developed for the control of insect pest. One reason for the limited commercialization of viruses is that they are usually produced in living insects, which is a complex, expensive, and labor-intensive process. Insect cell culture makes the *in vitro* production of viral pesticides possible. Generating insect viruses in cell culture systems has the advantages over *in vivo* systems of being highly controlled and reproducible. None of *Dentrolimus superans* cell lines has been established up to present. We propose to establish a cell line from *D. superans* in which CPV can replicate well, and to acquire fundamental knowledge on pathogenesis of the virus in the cell culture system for improving the character of the virus and for making mass production of the viral insecticides.

Materials and methods

Embryonated eggs

Embryonated eggs of the *Dendrolimus superans* were collected within 24 h of oviposition and held an additional 4 d at 27 ℃. At this time, the embryonated eggs were getting from deep green to light green in colour.

Primary cell culture

The intact eggs were immerged in a 2% sodium chlorite for 15 min and then washed for 10 min in 75% ethanol. After washing for three times in sterile water, the eggs were transferred to a mortar with 5 mL of TC-100 medium. The eggs were pulverized and 1 mL of the embryonic tissue was transferred to 25-mL flasks with 4-mL fresh medium in each. All of the flasks were incubated at 28℃ and half the medium was replaced weekly.

Subculturing

Subcultures were made when confluent cell sheets covered the bottom of the flasks about 16 months of initiation of the primary culture. The suspension with cells was obtained by gentle pipetting the bottom of the tissue culture flask, and removed to a flask with an equal volume of fresh medium. The flask was incubated at 28 ℃ .The interval between subcultures varied for the early cultures, but after 15 passages, subculturing was required weekly. The cell line was designated NEAU-Ds-95082 (Ds-950821).

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Karyotype analysis

The cells from the 50th passage of the tissue culture in logarithmic growth phase were treated with colchicine at a final concentration of 0.005 mg per mL for 3 h and allowed free with a rubber policeman and dispersed. The suspension was placed into a 15ml centrifuge tube and spun down, and the cells were processed according to the methods of schneider and Earley (Schneider 1973; Earley 1975) as modified. Twenty cells were counted under inverted phase microscope.

Biochemical marker.

The isozyme phenotype of EST (Esterase) from three cell lines was examined: NEAU-Ms-927311 (Ms-927311), NEAU-Xc-920730 (Xc-920730), and NEAU-Ds-950821 (Ds-950821). The isozyme was separated by polyacrylamide gel electrophoresis (PAGE) described by Brown and Knudson (Brown *et al* 1980). The mobility of the enzyme hands in the electrophorsis system was recorded. The cell line of *Spodoptera frugiperda* (SF-21AE) was chosen as the standard.

Preparation of the viral inoculum

The polyhedra of DsCPV were propagated in *D. superans* larvae and purified by gradient centrifugation of 40%-60% (w/v.) sucrose. The polyhedra were dissolved in 0.05M-NaCl-0.01M-Na_2CO_3 (pH 10.8) by stirring at 37℃for 10min. The pH was lowered by adding 1N-HCl to pH 8.0, and the sample was centrifuged at 1000 g for 10 min to remove undissolved polyhedra. The supernatant was mixed with 3×volume of TC-100 medium and then sterized by 0.22 μ membrane filter(Shen Hongjian *et. al.* 1994).

Virus susceptibility

25 cm^2-plastic flasks were seeded with 1.0×10^6 cells of 3 day-old suspension in 2 mL of TC-100 medium .The cells were allowed to attach for 2 h at 28℃ and the medium were removed. Sterile 2 mL aliquots of the viral inoculum were left to be absorbed for 2 h at 28℃and the cells were fed with 5-mL medium at 28℃ to test for virus susceptibility (Hink 1970; Li 1982).

Results

Growth of primary cultures

The early growth of the primary cultures followed the pattern generally observed in lepidoptera cell culture. Attachment of the vesides and tissues from embryonated eggs had began within 24 h and in the culture, clumpings proceeded rapidly during the first 48 h (Fig.1.A). Cell migration out of tissue fragments also occurred with fibroblast-like (Fig.1.B), and neuroblast-like (Fig.1.C) cells within 30 d after explantation. By the end of 2 months *in vitro* the bottoms of the flasks were nearly covered with networks of the culture (Fig.1.D) and the culture developed slowly until 15 months of explantation. After this interval the culture developed fast and the bottom of the flasks were nearly covered with layers of cells (Fig.2.A). At this time the first subculture of Ds-950821 cell line was obtained.

Morphology and growth

The morphological and growth characters of this cell line were observed after the 40th passage of subculture. The cell line consists of a mixture of cell types, including spherical shaped cells and spindle shaped cells (Fig.2.B). The cells firmly attached to the surface of the flasks. After culturing, there was an initial decline in the cell population within 24 h. Thereafter the cell population grew rapidly and peaked 144 h postseeding. The maximum cell population per mL and doubling time for the line were 2.2×10^6 cells/mL and 52.6 h respectively.

Karyology

The cell line showed the chromosome distribution pattern typically associated with cell lines obtained from Lepidoptera; the chromosomes of the cell line were condensed short rods and the numbers of chromosome ranged between 160 and 200.

Isozyme analysis

IPLB-SF-21AE cell line grown in TC-100 medium was used as internal standard .The initial and the most deeply stained band of the enzyme from the standard was rated as 100, then the mobility of the bands from other cell lines was determined. The result revealed distinctive patterns when compared with several other Lepidopteran cell lines (Table 1).

Table 1. Mobility of Esterase from insect cell lines

SF-21AE	Ms-927311	Xc-920730	Ds-950821
S. frugipera	*M. separata*	*X. c-nigrum*	*D. superans*
			60
	75		
		78	
			80
	88		
		90	
		95	
100		100	
		110	
115			

Virus susceptibility

The virus susceptibility determination of Ds - 950821 cell line was carried out at the 50th passage

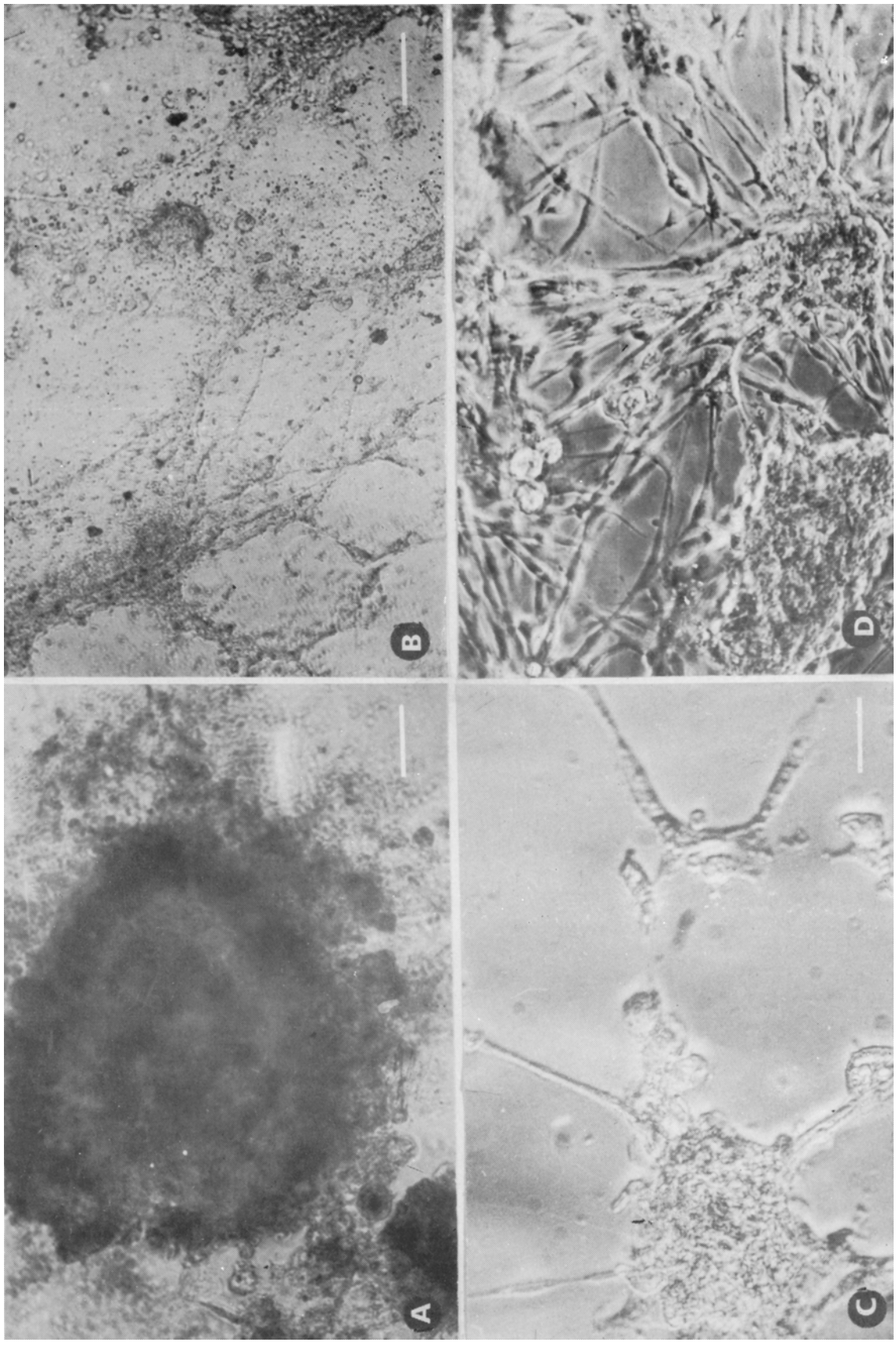
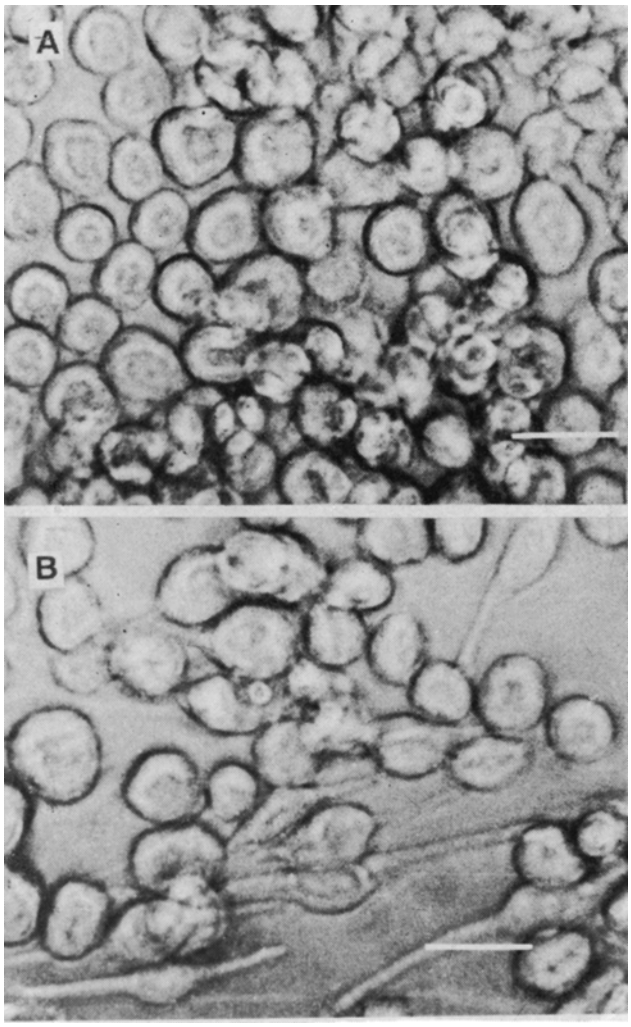


Fig. 1. Phase contrast micrographs on growth of primary culture during 15 months.
Maker bars equal 50 μm; all micrographs are at the same magnification.
A. Attachment of tissue and clumpings. B. Fibroblast-like cells. C. Neuroblast-like cells. D. The networks of tissue culture



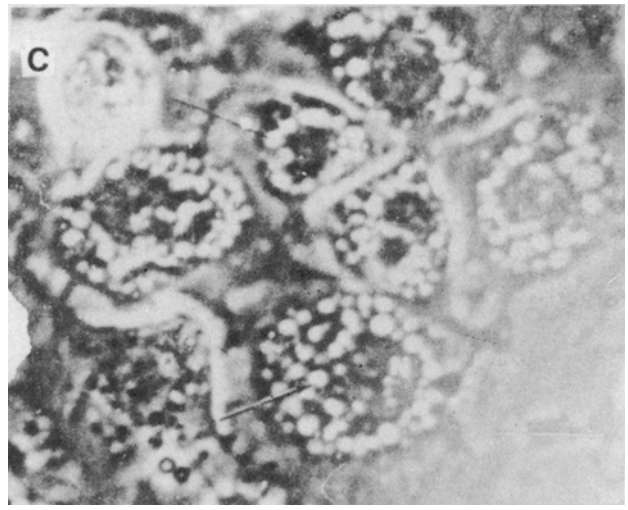


Fig. 2. Phase contrast micrographs on healthy and infected cells of NEAU-Ds-950821

A. The layer of culture cells before the first subculture;
B. B. 3 day-old healthy cells in 40th subculture including spherical and spindle shaped cells. Marker bars from A and B equal 25 μm;
C. Infected cells ,with refractive polyhedra (arrows). Marker bar equals 10 μm. N=nuclei.

of the cell line by inoculation with virions from *D. superans* cytoplasmic polyhedrosis virus (DsCPV). The result showed that the cytopathogenic effect was observed after incubation of 36 h and polyhedra were found 72 h postinfection (Fig.2.C) individually. About 55 percentage of infected cells was recorded.

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